Tetrandrine Works as a Major Component for the Immune-Modulatory Action of Fang-Ji-Huang-Qi-Tang: A Pharmacokinetics and Pharmacodynamics Study in Rats
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Purpose
Fang-Ji-Huang-Qi-Tang (FHT) is one of the classic formulas from Jin-Gui-Yao-Lue in China. This herbal mixture possesses potent immune-modulatory action. However, the pharmacokinetics of FHT and the major components for its pharmacodynamics are still unclear. The aim of this study is to investigate the pharmacokinetics of active components in FHT. The comparison of the different pharmacokinetic behaviors between pure tetrandrine (TET) and that in FHT at three different doses in Sprague-Dawley rats were also evaluated. In addition, a preliminary study on the mechanism of its immune-modulatory action was performed.

Methods
A rapid and sensitive UPLC-MS/MS method was developed for simultaneous quantification of tetrandrine, fangchinoline, magnoflorine, calycosin-7-glucoside, astragaloside IV, liquiritin and glycyrrhetic acid in rat plasma. Based on the developed method, the pharmacokinetics study was performed after the oral administration of FHT (2.67 g/kg, 4.00 g/kg and 6.00 g/kg) and pure TET (7.36 mg/kg, 11.03 mg/kg and 16.54 mg/kg), respectively. In the meantime, the effects of FHT on ConA-induced proliferous T lymphocytes were evaluated. The elucidation on the potential mechanism of the immune-modulatory action of FHT and TET was also performed.

Results
After the oral administration of FHT to rats, tetrandrine, fangchinoline, magnoflorine, calycosin-7-glucoside, astragaloside IV, liquiritin and glycyrrhetic acid were found to be the major components in the post-dosing rat plasma. Tetrandrine, fangchinoline and glycyrrhetic acid reached C max after 9 h with a long mean residence time and a slow elimination. Magnoflorine, calycosin-7-glucoside and liquiritin were rapidly absorbed with C max less than 1 h. In comparison to the pharmacokinetic parameters of pure compound TET, the AUC of TET in FHT was significantly increased (P<0.05), whereas the Clearance was significantly decreased (P<0.05), indicating that the other compounds in FHT may influence the absorption and elimination of TET. Meanwhile, the obvious inhibitory effect of FHT and TET on proliferative T lymphocyte, with significant downregulation of IL-17 and INF-γ level and upregulation of IL-10 level (p<0.01), was observed. Also, the phosphorylation level of STAT5 and expression level of Foxp3 of T lymphocyte were promoted with the increasing number of regulatory T cells (Treg cells), indicating that FHT and TET potentially inhibited the proliferation of T lymphocyte by promoting the augmentation and differentiation of Treg cells. TET, working as the major absorbed component into systemic circulation, may play an important role in this process.

Conclusion
A sensitive and validated UPLC-MS/MS method was firstly established for the simultaneous quantification of tetrandrine, fangchinoline, magnoflorine, calycosin-7-glucoside, astragaloside IV, liquiritin and glycyrrhetic acid in rat plasma after the oral administration of FHT. Alkaloids were the major components absorbed into systemic circulation. The bioavailability of TET could be improved by the other compounds in FHT. In addition, the increasing proliferation and differentiation of Treg cells were assumed to be the mechanism of FHT’s inhibitory effect on the proliferative T lymphocyte. TET played an important role in the immune-modulatory action of FHT.